Claims

1) An apparatus for dispensing a sample for analysis by electrospray ionisation mass spectrometry, said apparatus comprising a substrate of electrically insulating material, the substrate comprising at least two covered microstructures both having an outlet at the edge of the substrate where the electrospray is to be generated by application of a voltage and an inlet for fluid introduction, one of said microstructures containing the sample solution to be sprayed and at least one other of said microstructures containing a second fluid, preferably a sheath liquid or a sheath gas, characterized in that the sample solution and the second fluid are arranged to be directly mixed in the Taylor cone of the spray.

2) An apparatus according to claim 1 wherein said substrate is a multilayer body, preferably of polymer material(s), in which at least two layers of said multilayer body each comprise one of said at least two microstructures.

3) An apparatus according to claim 1 or 2, having a thickness smaller than 500 µm.

4) An apparatus according to any preceding claim, which comprises electrically or ionically conductive means for applying a voltage to the sample and/or sheath liquid solution(s), said conductive means having a controlled size and location.

5) An apparatus according to claim 4, wherein said conductive means comprises one or a plurality of electrodes and/or one or a plurality of electrically conductive pads.

6) An apparatus according to claim 5, wherein said conductive means is(are) integrated in one wall of said microstructure(s) and/or is in contact with the solution(s) at the inlet(s) of said microstructure(s).

7) An apparatus according to any one of claims 1 to 3, wherein the spray voltage is applied through external electrically or ionically conductive means arranged to be in contact with the solutions to be sprayed, for instance by placing

said conductive means in the solutions to be sprayed at the inlets of said microstructures.

8) An apparatus according to any of claims 4 to 7, wherein said conductive means comprises an electrically conductive ink, or a metallic layer, or a conducting polymer such as e.g. polypyrrole or polyaniline, or a conductive gel, or an ion exchange polymer arranged to be in contact with the solutions to be sprayed.

9) An apparatus according to any preceding claim, wherein the distance between the outlet of the sample microstructure and that of the sheath liquid microstructure is smaller than 200 μm.

10) An apparatus according to claim 9, wherein the sample microstructure and the sheath liquid microstructure are connected at the edge of the substrate, thereby forming a single outlet.

11) An apparatus according to any preceding claim wherein said microstructure outlets taper in the spraying direction.

12) An apparatus according to any preceding claim wherein the microstructure outlets are hydrophobic or are surrounded by a hydrophobic material.

13) An apparatus according to any preceding claim, wherein said microstructures have at least one dimension of less than about 150 μm.

14) An apparatus according to any preceding claim, wherein said sample microstructure and/or said sheath liquid microstructure communicate(s) with a network of microstructures.

15) An apparatus according to any preceding claim, wherein said sample microstructure has a hydrophilic surface.

16) An apparatus according to any preceding claim, wherein said covered microstructures are sealed by gluing, lamination or pressure application of a polymer foil.

17) An apparatus according to any preceding claim, wherein said sample solution is an aqueous solution.

18) An apparatus according to any preceding claim, wherein said sample microstructure contains a biological or a chemical material, such as e.g. proteins, enzymes, antibodies, antigens, sugars, oligonucleotides, DNA, cells or an organic compound, which is filled in said microstructure or which is coated, immobilized or covalently bound to the microstructure surface or to a solid support (such as a membrane, a gel, a sol-gel, beads or the like), so as to perform a biological assay such as enzymatic, affinity, activity, immunological and/or cellular assays and/or to perform a chemical assay such as solubility, permeability or lipophilicity tests and/or to perform enzymatic or chemical digestion, sample derivatisation or electrochemically induced reactions such as protonation, tagging using quinones or any other redox reactions.

19) An apparatus according to any preceding claim, wherein said sample microstructure comprises a separation means, comprising at least one of a solid phase, a chromatography medium or a capillary electrophoresis system.

20) An apparatus according to claim 19, wherein said separation means comprises a solid phase selected from a membrane, beads and/or a section of the microstructure wall.

21) An apparatus according to any preceding claim, wherein said sample microstructure is connected to a separation means, e.g. a chromatography column, an electrophoresis unit, a membrane, a desalting step, an affinity column or the like.

22) An apparatus according to claim 21, wherein said sample microstructure, preferably comprising a network of interconnected microstructures, is used to collect fractions from said separation means and further dispense them or part of them into the mass spectrometer by electrospray generation.

23) An apparatus according to any preceding claim, which is supported in a device for the precise positioning of the microstructure outlet in front of a mass

spectrometer entrance and/or the facilitation of the electrical connection(s) with one or a plurality of power supplies and/or the introduction of the sample and/or sheath liquid solution(s) with minimized dead volume.

24) An apparatus according to any preceding claim, wherein a third microstructure is used to introduce a sheath gas in the spray.

25) A method of dispensing a sample for subsequent analysis by electrospray mass spectrometry using the apparatus of any one of claims 1 to 24, comprising the steps of applying a voltage to the sheath liquid solution in order to initiate the spray and of imposing another voltage to the sample solution in order to induce a flow of sample, both sheath liquid and sample solutions being mixed directly in the Taylor cone.

26) A method according to claim 25, wherein the proportion of sheath liquid and of sample solutions sprayed is controlled by the difference of the voltage applied in the sheath liquid and that applied in the sample solution.

27) A method according to claim 25 or 26, wherein a floating voltage is applied between the sample solution and the sheath liquid.

28) A method according to claim 27, wherein an aqueous sample solution is sprayed.

29) A method according to any one of claims 25 to 28, comprising introducing a compound of known concentration in either or both of the sample and/or the sheath liquid solutions.

30) A method according to claim 29, comprising the step of controlling the proportion of sheath liquid and sample solution sprayed and/or of performing quantitative mass spectrometry analyses.

31) A method according to any one of claims 25 to 30, comprising immobilizing molecules of the sample reversibly on a solid support, and releasing said molecules

from the solid support into the sample microstructure by a spraying buffer or by a gradient of different solvents.

32) A method according to any one of claims 25 to 31, comprising the step of filling said sample microstructure with, or immobilizing or covalently binding to the surface of said microstructure or to a solid support (such as a membrane, a gel, a sol-gel, beads or the like), a biological or a chemical compound, such as e.g. proteins, enzymes, antibodies, antigens, sugars, oligonucleotides, DNA, cells or an organic compound, so as to perform a biological assay such as enzymatic, affinity, activity, immunological and/or cellular assays and/or to perform a chemical assay such as solubility, permeability or lipophilicity tests and/or to perform enzymatic or chemical digestion, sample derivatisation or electrochemically induced reactions such as protonation, tagging using quinones or any other redox reactions, with subsequent analysis by electrospray mass spectrometry.

33) A method according to claim 32, wherein at least one affinity agent is immobilized on said solid support, said affinity agent being selected from antibodies, antigens, oligonucleotides, DNA strains and the like.

34) A method according to claim 32 and 33, wherein after the step of immobilizing the molecules of the sample, the solid support is placed in contact with the sample microstructure.

35) A method according to claim 31, wherein a chemical reaction and/or an affinity reaction occurs in or on said solid support prior to the releasing step.

36) A method according to claim 35, wherein said chemical reaction and/or affinity reaction comprises at least one of desalting, enzyme or chemical digestion, chemical transformation and purification.

37) A method according to any one of claims 32 to 36, wherein said solid support is selected from polymers, ceramics, metallic and glass materials; e.g. polyvinylidenefluoride (PVDF), nitrocellulose, cellulose acetate, acrylamide, agarose, or the like.

38) A method according to claim 32, wherein after the step of coating a compound in the sample microstructure, a buffer is introduced to partially or totally dissolve said compound for subsequent analysis by electrospray mass spectrometry.

39) A method according to any one of claims 32 to 38, wherein a separation is performed in the sample microstructure and/or an partial or total extraction in a solution in contact with the sample solution is performed prior to spraying of the sample solution.

40) A method according to any one of claims 32 to 39, wherein an organic phase is deposited at the inlet of the sample microstructure in order to avoid evaporation of the sample solution to be sprayed.

41) A method according to any one of claims 32 to 40, wherein the sample and sheath liquid solutions are applied directly in the inlet reservoirs of the respective microstructures and sprayed into a mass spectrometer, without application of an external force (e.g. back pressure).

42) A method of fabricating an apparatus for dispensing a sample for subsequent analysis by mass spectrometry, comprising the steps of taking a substrate of electrically insulating material, fabricating at least two covered microstructures, both having an outlet at the edge of the substrate where the spray is to be generated by application of a voltage and an inlet for fluid introduction, so that the sample and sheath liquid solutions to be sprayed from the microstructures through these outlets are mixed in the Taylor cone.

43) A method of fabricating an apparatus according to claim 42, comprising the step of taking a substrate which is a multilayer body, fabricating at least one covered microstructures in a plurality of layers, assembling said plurality of layers and optionally cutting the assembled multilayer body, so as to obtain at least two covered microstructures, both having an outlet at the edge of the substrate where the spray is to be generated by application of a voltage and an inlet for fluid introduction, so that the sample and sheath liquid solutions to be sprayed from the microstructures through these outlets are mixed in the Taylor cone.

44) A method according to claim 42 or 43, comprising the step of integrating electrically or ionically conductive means for applying a voltage to the sample and/or sheath liquid solution(s), said conductive means having a controlled size and location.

45) A method according to any one of claims 42 to 44, wherein said conductive portion means is formed by laser photoablation, by plasma etching, by chemical etching, by deposition of an ink, of a conductive polymer, by integration of an ion exchange material, by metal deposition, by sputtering or the like.

46) A method according to any one of claims 42 to 45, wherein said conductive means is integrated in the cover of the microstructures.

47) A method according to any one of claims 42 to 46, comprising adding an electrode in a reservoir connected to the inlet of at least one of the covered microstructures, such as to apply a voltage from outside the microstructure(s).

48) A method according to any one of claims 42 to 47, wherein the substrate is a polymer material.

49) A method according to any one of claims 42 to 48, wherein the microstructures are formed by laser photoablation, UV-Liga, embossing, injection molding, solvent casting, light or thermal induced polymerization, silicon technology or superposition of layers at least one comprising mechanically drilled grooves, hollows or holes.

50) A method according to any one of claims 42 to 49, wherein a plurality of apparatuses are fabricated in the same substrate, thereby creating an array of apparatuses.

51) A coupling device comprising one or a plurality of apparatus(es) according to any one of claims 1 to 24, further comprising one or a plurality fluid connection(s) for minimizing dead volumes at the microstructure inlets, and/or electrical connection(s) for application of potential differences in the microstructures

and/or a system enabling the precise positioning of the apparatus(es) in front of a mass spectrometer entrance.

52) An analytical instrument comprising an array of apparatuses, each according to any one of claims 1 to 24.

53) A method of analyzing a plurality of samples, comprising taking an array of apparatuses, each according to claim 1 to 24, using a plurality of the apparatuses in turn to collect a sample, and dispensing each sample from the respective apparatus, and analyzing each sample by mass spectrometry.

54) A method according to claim 53, wherein said samples are collected from an analytical system, e.g. a chromatograph, an electrophoretic unit, a separation unit or an affinity system.

55) A method of performing chemical or biological assay using one apparatus or an array of apparatus, each according to claims 1 to 24, with detection by electrospray mass spectrometry.

56) A method according to claim 55 wherein said chemical or biological assays are selected from enzymatic, affinity, activity, immunological and/or cellular assays, solubility, permeability or lipophilicity tests.